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REGULAR PAPER

Sensitivity to high temperature and water stress in recalcitrant *Baccaurea ramiflora* seeds

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Abstract Southeast Asia experiences one of the highest rates of deforestation in the tropics due to agricultural expansion, logging, habitat fragmentation and urbanization. As tropical rainforests harbour abundant recalcitrant-seeded species, it is important to understand how recalcitrant seeds respond to deforestation and fragmentation. *Baccaurea ramiflora* is a recalcitrant-seeded species, widely distributed in Southeast Asian tropical rainforest. In this study, *B. ramiflora* seeds were sown in three plots, one in a nature reserve and two in disturbed holy hill forests, to investigate seed germination and seedling establishment in the field, while laboratory experiments were conducted to investigate the effects of high temperature and water stress on germination. It was found that seed germination and seedling establishment in *B. ramiflora* were clearly reduced in holy hills compared to the nature reserve, although the seeds were only moderately to minimally recalcitrant. This was potentially caused by increased temperature and decreased moisture in holy hills, for laboratory experiments showed that seed germination was greatly inhibited by temperatures ≥35 °C or water potentials ≤−0.5 MPa, and depressed by heat treatment at 40 °C when the continuous heating period lasted for 240 h or daily periodic heating exceeded 10 h. Unlike orthodox seeds, which can endure much higher temperatures in the air-dried state than in the imbibed state, both blotted and immersed *B. ramiflora* seeds lost viability within a narrow temperature range between 50 and 60 °C. As recalcitrant seeds can be neither air-dried nor heated, species producing recalcitrant seeds will suffer more than those producing orthodox seeds in germination and seedling establishment from increased temperature and decreased moisture in fragmented rainforests, which results in sensitivity of recalcitrant-seeded species to rainforest fragmentation.

Keywords High temperature stress · Rainforest fragmentation · Recalcitrant seeds · Seed germination · Species shift · Water restriction

Introduction

Deforestation and fragmentation continue to be a serious threat to tropical rainforests due to land-use change and logging (Li et al. 2009; Sodhi et al. 2010; Tollefson 2015), which causes losses of nearly 7000 km^2 of tropical rainforests each year in the Amazon alone (Davidson et al. 2012). The situation is even more serious for Asian tropical rainforests, for tropical Asia has been experiencing some of the highest deforestation rates observed across the tropics though exact data is not unknown at present (Achard et al. 2002; Laurance 2007; Liu and Silk 2014; Nghiem et al. 2015). Taking measurements to protect tropical rainforest is not only necessary but also urgent.

Understanding what kinds of plant species are more sensitive to fragmentation is helpful for conservation of tropical rainforests. As tropical rainforests have numerous species with recalcitrant seeds, the response of recalcitrant-seeded species to fragmentation is a crucial issue. Roberts (1973) defined recalcitrant seeds as those that are sensitive to drying and freezing, and thus hard to store in conventional seed banks (Roberts and King 1980). Most

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recalcitrant seeds originate from tropical and subtropical areas (Farnsworth 2000; Tweddle et al. 2003). In tropical rainforests species producing recalcitrant seeds are frequent and dominant: they are estimated to make up as much as 30 % of the species in tropical rainforests in Xishuangbanna, SW China, according to our personal observations.

Baccaurea ramiflora Lour. is a common understory tree species native to Southeast Asian tropical rainforests (Cao et al. 2008; Wu et al. 2008), which produces recalcitrant seeds (Wen and Cai 2014). In a previous study, Wen and Cai (2014) found that germination of *B. ramiflora* seeds was significantly delayed in non-rainforest conditions compared with the rainforest understory, and greatly reduced in an open site. These authors suggested that *B. ramiflora* had a high dependence on intact rainforests for seed germination and seedling establishment, and that this can be attributed to the sensitivity to high temperature and desiccation of its seeds. The increased temperature and decreased moisture availability that result from fragmentation and disturbance decrease the probability of seed germination and seedling establishment. These results provided a possible explanation for the frequently-reported decline of large-seeded and old-growth taxa in fragmented rainforests (Laurance et al. 1998a, 1998b, 2006; Zhu et al. 2004, 2010).

However, high temperature and water stress may occur in different ways. Temperature stress, for example, can occur briefly or over a long period, continuously or intermittently, be experienced as atmosphere temperature or ground temperature, and as increased mean temperature or maximum temperature, or a lengthened warm period. To identify what is more stressful is useful to enrich our knowledge about the effects of fragmentation on germination of recalcitrant seeds. In this study, *B. ramiflora* seeds were sown in three plots, one in a nature reserve and two in holy hill forests, while laboratory experiments were conducted to investigate seed germination under high temperature and water stress in more detail, with the aim of improving our understanding of why and how fragmentation causes species composition changes in rainforests.

Materials and methods

Seed collection

Baccaurea ramiflora produces large amounts of fruits in Xishuangbanna during July to August every year. Seeds used in the experiments were collected in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (21°55′ N, 101°15′ E), in 1999 (for field study), 2012 and 2014 (for laboratory study). After collection, seeds were extracted from fruits, cleaned manually and blotted dry, and then seed weight, initial moisture content and viability were determined. The remaining seeds were stored in plastic bags at 15 °C for a maximum of 3 days before use.

Field study

Three plots of tropical rainforests in Xishuangbanna, were chosen to carry out this experiment, among them, Chengzi and Manyangguang are ‘holy hills’, i.e., remnants of tropical rainforests protected by the Dai ethnic group for religious reasons. The third, Menglun ‘55 km’, is located in the Menglun section of the Xishuangbanna National Nature Reserve, close to the 55-km milestone, and was chosen as a control. These three plots have similar latitude and climate, but Menglun ‘55 km’ has primary forest with very slight disturbance because the reserve was established in 1958, while the forests in Chengzi and Manyangguang, which are adjacent to villages and receive no legal protection, were evaluated as slightly and very seriously disturbed, respectively, although Manyangguan is relatively large (Zhu et al. 2004). These two holy hills had been isolated for many decades before this experiment was conducted, and increased temperature and decreased moisture have been recorded for both (Ma et al. 1998; Zhu et al. 2004), and a significant shift in floristic composition reported in Manyangguang (Zhu et al. 2010).

In each plot, an area of forest with similar canopy cover was chosen to set up three 1 m × 1 m quadrats. After removing covering herbs and litter, 100 freshly-harvested seeds were sown in each quadrat in August 1999, and covered by a metal net to exclude seed predators. In the following 2 months germination of these seeds was investigated once a month, and seedling survival was investigated once every 2 months until the rainy season began in June 2000.

Laboratory study

Effects of desiccation on seed moisture and viability

Two methods were used to dry the seeds. For rapid drying, seeds were mixed with plenty of activated silica gel, which was regularly changed. For slow drying, seeds were placed in a monolayer in an air-conditioned room (25 ± 2 °C, 70–85 % RH). In both methods seeds were regularly sampled for moisture content and viability determination.

High-temperature tolerance of fresh quiescent seeds

The method previously described by Wen et al. (2015) was employed to investigate the tolerance of quiescent seeds to high temperature. A water bath was used to create a temperature range from 30 to 95 °C at 2.5 °C increments. Seeds in a monolayer in dishes were exposed to a temperature for 30 min, either in a blotted-dry state, or immersed in water at the same temperature as that in the water bath. After heat treatment, the seeds were removed from the water bath and immediately sown on agar for viability determination.

Effects of incubation temperature on seed germination

Fresh seeds were sown on 1 % plain agar in petri dishes and placed in incubators set at constant temperatures of 10, 15, 20, 25, 30, 35 and 40 °C, and a fluctuating day/night temperature of 18/28 °C. A 12-h photoperiod of 25 μmol m^{-2} s^{-1} irradiance was provided by a white fluorescent light.

Effects of water availability on seed germination

Osmotic solutions with water potentials from −0.1 to −1.0 MPa were created using polyethylene glycol (PEG) 8000 and NaCl, respectively. Water potentials of these solutions at 25 °C were calculated according to the equations described by Michel (1983) and Lang (1967). De-ionized water was included as a control (0 MPa). The seeds were sown in glass jars sealed with breathable film and placed in an air-conditioned room (25 ± 2 °C, 70–85 % RH) for germination. Defatted cotton moistened with 10 ml treatment solution was used as germination medium, which was refreshed twice a week.

Effects of continuous heat treatment on seed germination

In this experiment, 40 °C was chosen as the heat treatment temperature to investigate the response of *B. ramiflora* seeds to high temperature stress, because this has been reported as maximum ground temperature in a rainforest canopy gap in Xishuangbanna (Liu et al. 2000). Seeds sown on 1 % plain agar in petri dishes were placed in an incubator at 40 °C for up to 240 h. After heating, they were sampled, withdrawn from the incubator, and placed under ambient conditions (air-conditioned at 25 ± 2 °C and normal light) to check seed viability.

Effects of periodic high temperature on seed germination

This experiment was designed to investigate the response of *B. ramiflora* germination to periodic heat treatment. For this purpose, seeds sown on 1 % agar were physically transferred into and out of a 40 °C incubator so that they were exposed to daily alternations of 1 h/23 h, 2 h/22 h, 3 h/21 h, 5 h/19 h, 7 h/17 h, 9 h/15 h, 12 h/12 h and 15 h/9 h between 40 °C and ambient temperature (air-conditioned at 25 ± 2 °C).

Seed moisture content determination

The low temperature oven method for oily seeds recommended by ISTA (2006) was used, i.e., seed moisture contents were determined gravimetrically before and after drying for 17 ± 1 h at 103 °C and expressed as the mean ± SE of 8 replicates of single seeds on a fresh weight basis.

Seed viability and germination assessment

In this study, 20 seeds × 6 replicates were used for each treatment in all laboratory experiments. Petri dishes containing 1 % agar were used as germinators, except in the water stress experiment, where defatted cotton in sealed glass jars was specified in the methods. Seed germination was monitored once a week, and those with the radicle protruding to 1 cm were recorded as germinated, or survived for stress-treated seeds, and they were recorded as emerged when forming seedlings. After 2 months incubation all experiments finished and a crush test was applied to confirm that the ungerminated seeds were nonviable, with two exceptions. One was that some of the seeds incubated at 10 °C in the incubation temperature experiment kept firm but not germinated, or germinated but did not emerge. These were transferred to 25 °C for further germination. Similarly, the seeds treated with solutions of <−0.3 MPa were rinsed with deionized water after 2 months incubation and incubated on defatted cotton moistened with deionized water.

Data analysis

Data are presented as means and standard errors, and subjected to one- or two-way ANOVA and Duncan's multiple comparison tests (α = 0.05) after arc-sine transformation, using SPSS 13.0 for Windows. Probit analysis was employed to calculate moisture contents corresponding to 85 % viability as critical moisture contents.

Results

The seed lots used in different experiments exhibited some difference in traits, with the 100-seed weight ranging from 28.25 to 36.18 g, and initial moisture content from 56 to 60 %, but all had initial germination of over 95 %.

Seed germination and seedling establishment in fragmented rainforests

Most *B. ramiflora* seeds sown in the three plots germinated, but those sown in Menglun '55 km' had a final percentage germination of 90.7 ± 1.4 %, which is much higher than at Manyangguan (72 ± 2.4 %). Furthermore, germination was delayed in the two holy hills compared to the nature reserve (Table 1). Most seedlings at the holy hill sites did

Table 1 Seed germination and seedling establishment in three rainforest fragmentations in Xishuangbanna, Southwest China

	Menglun '55 km'	Chengzi	Manyangguang
Seed germination (%)			
In Sep, 1999	88 ± 3.7	75 ± 2	55.3 ± 5
In Oct, 1999	90.7 ± 1.4	85.7 ± 1.4	72 ± 2.4
Seedling establishment (%)			
In Dec, 1999	80 ± 3.3	67.3 ± 1.7	59.3 ± 5.4
In Feb, 2000	66.7 ± 2.8	50.7 ± 1.7	39.7 ± 3.7
In Apr, 2000	37.7 ± 1.7	31.3 ± 4.3	17 ± 2.9
In Jun, 2000	26.7 ± 5.7	20 ± 2.9	7.7 ± 1.7

Values are mean ± SE of 3 replicates of 100 seeds

not survive the dry season, showing that Menglun '55 km' is more suitable for seedling establishment than holy hills. In June 2000, mean seedling number established from 100 seeds was 26.7 ± 5.7 in Menglun '55 km', 20 ± 2.9 in Chengzi and 7.7 ± 1.7 in Manyangguan (Table 1). The differences between the plots were significant for both seed germination ($P < 0.001$) and seedling establishment ($P < 0.05$).

Effects of desiccation on seed moisture and viability

Distinct desiccation behaviors were found under different drying regimes. In the silica drying experiment, seeds lost moisture rapidly, showing a linear desiccation time course, with the seed moisture reaching 6 % after 96 h. Under air-drying, seeds also progressively lost moisture, but at a relatively slower rate, with moisture reaching 23.4 % after 108 h. Before this point desiccation was also linear, but after this there was little change, because seed moisture had reached equilibrium with the ambient conditions (Fig. 1a).

The seeds were sensitive to desiccation, but exhibited different sensitivities under silica- and air-drying. Air-dried seeds demonstrated better desiccation tolerance than those that were silica-dried. This was especially obvious in the 32–47 % moisture range. Desiccation to 47 % moisture caused marked viability loss in silica-dried seeds, and there was further loss with increasing desiccation, while air-dried seeds could be dried to 32 % without substantial viability loss (Fig. 1b). Probit analysis indicated that the moisture content corresponding to 85 % emergence was 46.91 and 31.55 % for silica- and air-dried seeds, respectively. When desiccated below 32 %, the air-dried seeds had an abrupt decrease in both survival and emergence, and both silica- and air-dried seeds completely lost viability around 18 % moisture content (Fig. 1b). Silica-dried seeds had their seed coats ruptured from the beginning of the desiccation experiment while this did not happen to air-dried seeds (Fig. 2).

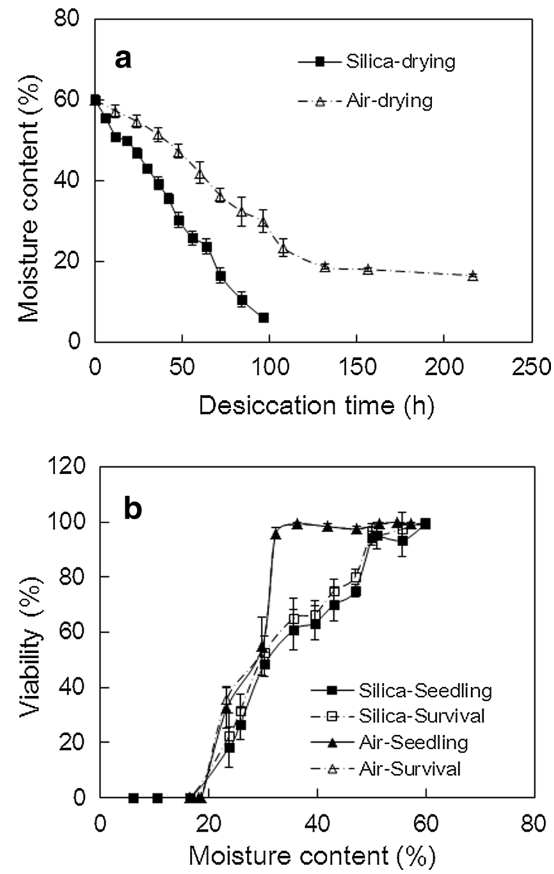

Fig. 1 Effects of air- and silica-drying on seed moisture content (**a**) and viability (**b**). Moisture contents are expressed as mean ± SE of 8 replicates of single seeds, and percentage survival and seedling values as mean ± SE of 6 replicates of 20 seeds. Seeds collected in 2014

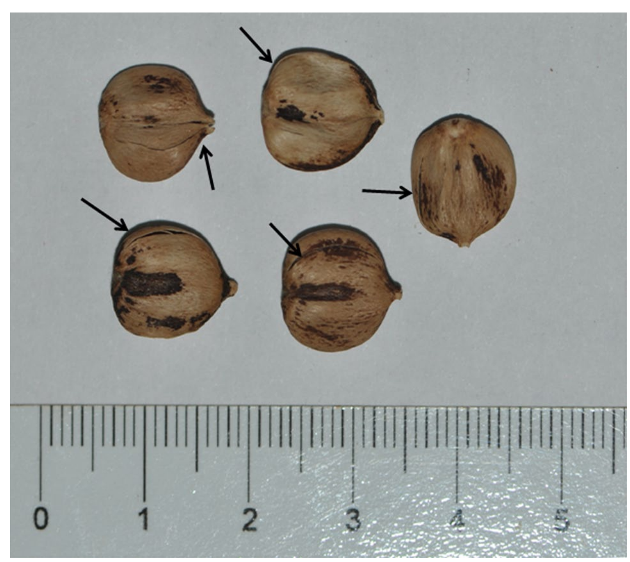

Fig. 2 Seeds with their coats ruptured during drying by silica gel, with cracks indicated by arrows

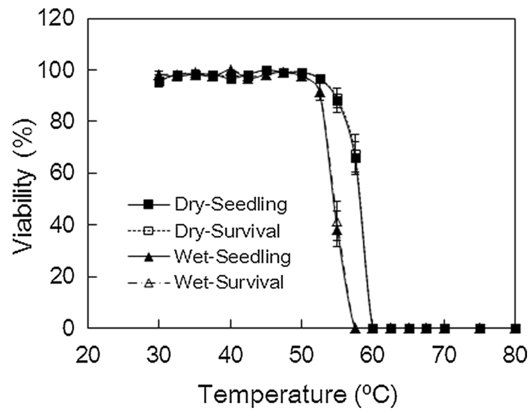

Fig. 3 Effects of half an hour heat shocks from 30 to 80 °C on viability of blotted-dry (Dry) or immersed (Wet) seeds. Percentage survival and seedling values are expressed as mean ± SE of 6 replicates of 20 seeds. Seeds collected in 2014

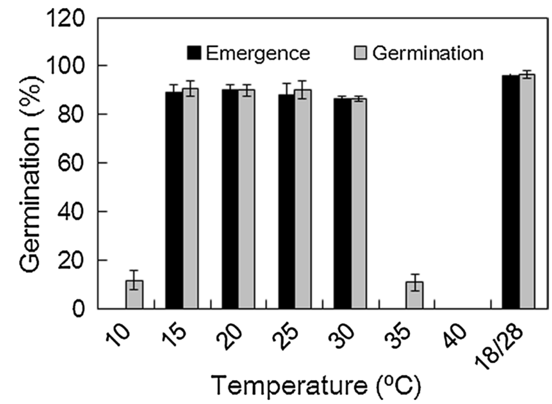

Fig. 4 Effects of incubation temperatures on seed germination. Seeds were incubated at constant temperature from 10 to 40 °C, and fluctuating temperature of 18/28 °C. Percentage germination and seedling values are expressed as mean ± SE of 6 replicates of 20 seeds. Seeds collected in 2012

High-temperature tolerance of fresh quiescent seeds

Half an hour's heat treatment reduced the viability of both blotted and immersed seeds in the temperature range from 50 to 60 °C (Fig. 3, with data not shown for treatment temperature >80 °C). Below this range, heat treatment for 30 min had little or no impact on either, while above this range it was lethal, with no survivors from either treatment. Within this temperature range, blotted seeds had a higher tolerance of short-term temperature shock than immersed seeds. At 55 °C, emergence of blotted and immersed seeds was 90 and 40 %, respectively. Two-thirds of blotted seeds survived at 57.5 °C, but all immersed seeds were killed at this temperature (Fig. 3). Two-way ANOVA found that the differences between blotted and immersed seeds were very significant ($P < 0.001$ for temperature, seed state, and temperature × seed state, for survival and emergence).

Effects of incubation temperatures on seed germination

Over 85 % of seeds germinated at constant temperatures from 15 to 30 °C, and fluctuating temperatures of 18/28 °C, and most germinated seeds developed into morphologically normal seedlings. Neither emergence nor germination percentage were significantly different among seeds incubated at 15, 20, 25 and 30 °C (Fig. 4), although the higher the incubation temperature, the faster the seeds germinated (data not shown). However, emergence and germination were significantly lower at constant temperatures than fluctuating temperature of 18/28 °C ($P < 0.001$). Germination was completely inhibited at 40 °C. When the experiment finished after 2 months, most seeds incubated at 35 °C had decayed, while 10 % had radical protrusion but failed to form seedlings. Only 10 % of seeds showed radical protrusion at 10 °C also, but in this case the ungerminated seeds were still firm, and germinated and emerged after being transferred to 25 °C, with a final emergence percentage of 70 %.

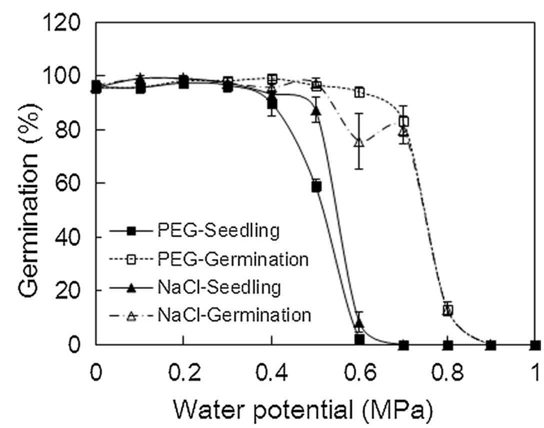

Fig. 5 Effects of water availability on seed germination. Water potentials were created by PEG 8000 and NaCl, respectively. Percentage germination and seedling values are expressed as mean ± SE of 6 replicates of 20 seeds. Seeds collected in 2014

Effects of water availability on seed germination

Both water potential and osmoticum made a difference to germination (for emergence, $P < 0.001$ for water potential, $P = 0.006$ for reagent and $P = 0.021$ for water potential × reagent), and variation was observed mainly between −0.4 and −0.8 MPa (Fig. 5). Compared with the control (deionized water, 0 MPa), water stress less than −0.4 MPa had no effect on germination, while germination was completely inhibited when water stress increased to −0.9 MPa. As water stress increased from −0.4 to −0.8 MPa, first

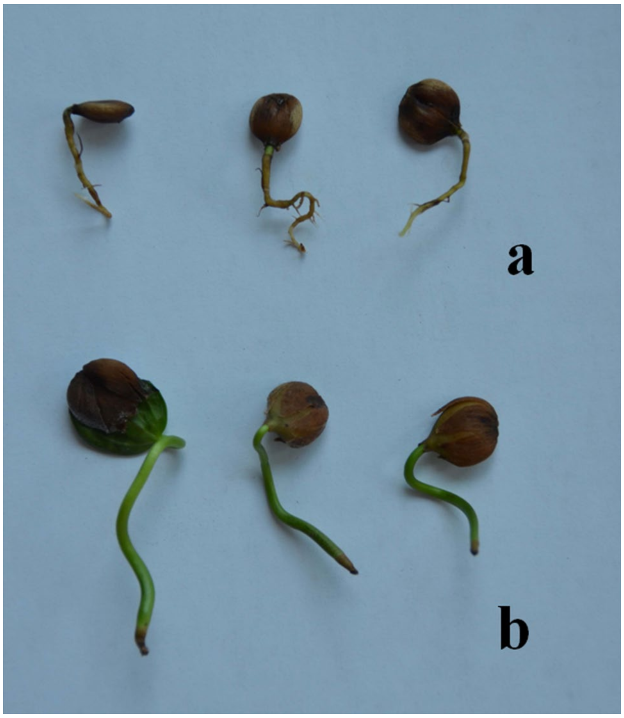

Fig. 6 Abnormal seedlings from germination under water stress of −0.6 MPa created by PEG 8000 (**a**) and NaCl (**b**). Seeds collected in 2014

emergence percentage decreased and then germination percentage. The emergence percentage was much lower than the germination percentage at the same water potential within this range, meaning that many seeds germinated but failed to form seedlings. *Baccaurea ramiflora* seeds are more sensitive to water stress created by PEG than by NaCl: the emergence percentage at −0.5 MPa was 60 and 90 % under PEG- and NaCl-stress respectively. Emergence and germination were intensively inhibited at −0.6 and −0.8 MPa, respectively, for PEG and NaCl solutions (Fig. 5). Ungerminated seeds remained firm, however, and most germinated after removal from stress (data not shown). Root growth was seriously inhibited but hypocotyls were less affected in seeds treated by NaCl solutions, but the opposite occurred in seeds treated by PEG solutions (Fig. 6).

Effects of continuous heat treatment on seed viability

Although *B. ramiflora* seeds cannot germinate in a constant temperature of 40 °C, they can withstand incubation at this temperature for shorter periods. Up to 12-h treatment had no significant effect on either emergence or survival, compared with the unheated control, but from 24 to 196 h the emergence and survival percentage declined gradually. An abrupt viability loss was caused by 240 h heat treatment, with only half the seeds surviving (Fig. 7).

Effects of periodic high temperature on seed germination

Compared to continuous heat treatment, *B. ramiflora* seeds were more sensitive to periodic high temperatures. The time period of daily heat treatments had significant effects on germination and emergence ($P < 0.001$ for both emergence and germination). All treatments were significantly different from the unheated control. Two to seven hours daily heat treatment at 40 °C depressed emergence percentage below 80 %. As the length of the daily high temperature period increased, emergence and germination percentage gradually decreased, and only few seeds emerged under a daily 15-h heat treatment (Fig. 8).

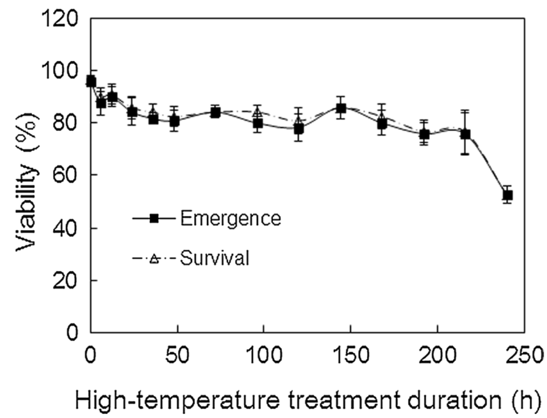

Fig. 7 Effects of continuous high temperature stress on seed viability. Seeds were heat-shocked at 40 °C for indicated period of time, and incubated at ambient conditions after release from stress. Percentage survival and seedling values are expressed as mean ± SE of 6 replicates of 20 seeds. Seeds collected in 2012

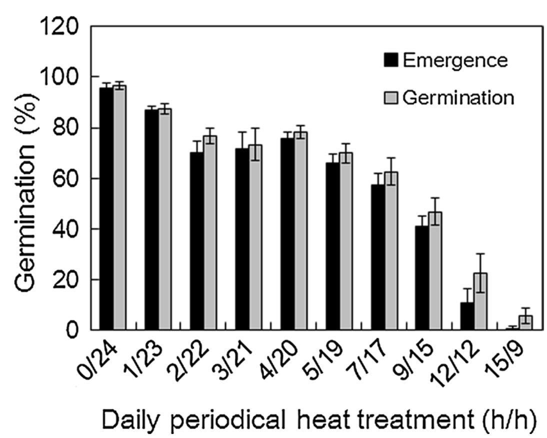

Fig. 8 Effects of daily periodic high temperature stress on seed germination. 40 °C high temperature and ambient temperature (h/h) were imposed on seeds alternately. Percentage germination and seedling values are expressed as mean ± SE of 6 replicates of 20 seeds. Seeds collected in 2012

Discussion

According to this and previous studies (Liu et al. 2014; Wen and Cai 2014; Yu et al. 2008), it was confirmed that *B. ramiflora* produces recalcitrant seeds, but they are only moderately to minimally recalcitrant (Farrant et al. 1988), since a comparison between rapid- and slow-drying treatments in this study found that slow drying enhanced desiccation tolerance (Fig. 1). Moreover, the impacts of low temperatures and moderate water stress were reversible in most seeds (Figs. 4, 5). In accordance with this, a recent study reported that CO treatment induced chilling tolerance in *B. ramiflora* seeds (Bai et al. 2012). These results help explain the finding in the previous report (Wen and Cai 2014) that germination of *B. ramiflora* seeds in non-rainforest conditions was only delayed, although greatly reduced in clearings, and also explain the wide distribution of *B. ramiflora* in rainforests in Xishuangbanna (Cao et al. 2008; Wu et al. 2008).

Though climax species and recalcitrant-seeded species are different conceptions, recalcitrant seeds are usually closely related with climax species while pioneer species usually produce orthodox seeds. In tropical rainforests there is a high proportion of climax species producing recalcitrant seeds, which are large in size, sensitive to drying, have high moisture contents when shed, germinate rapidly and do not enter a persistent soil seed bank (Farnsworth 2000; Tweddle et al. 2003). Deforestation and fragmentation cause microclimate changes in fragmented rainforests, including increased light and temperature, and decreased moisture, with the amplitude of the changes depending on disturbance extent and proximity of the forest edge (Camargo and Kapos 1995; Davies-Colley et al. 2000; Ma et al. 1998; Williams-Linera 1990). These microclimate changes resemble what occurs during retrogressive succession, so it can be expected that rainforest fragmentation will favour regeneration of orthodox-seeded species while disfavouring regeneration of recalcitrant-seeded species.

The present and previous (Wen and Cai 2014) studies aimed to link climate changes and species shifts in fragmented rainforests through the different responses of orthodox and recalcitrant seeds to increased temperature and decreased moisture. This study investigated seed germination and seedling establishment of *B. ramiflora* in three rainforests with different degrees of disturbance, while the previous one studied seed germination in habitats with different deforestation. At the same time, in order to understand responses of seed germination and seedling establishment to forest fragmentation and deforestation, laboratory experiments were carried out to characterize germination of recalcitrant *B. ramiflora* seeds under high temperature and water stresses. The previous study used graded temperature in different incubators and graded relative humidity created by saturated salt solutions to mimic the increased temperature and decreased moisture in rainforest fragments, testing mainly the effects of high temperature and water stress on seed moisture and viability. This study through increasing continuous or daily periodical heating treatment time at 40 °C, and decreasing water potentials of germination medium by PEG or NaCl, simulated the effects of high temperature and water stress on germination of *B. ramiflora* seeds. The laboratory and field experiments support each other, and together provide evidence for our hypothesis that deforestation and fragmentation put more pressure on germination and seedling establishment in this species because of the sensitivity of its seeds to increased temperature and decreased moisture.

High temperature plays an important ecological role, as shown by Chauhan and Johnson (2008a, 2008b, 2009), and Bear et al. (2012), and high-temperature tolerance may be a critical factor to determine different responses in recalcitrant and orthodox seeds to rainforest fragmentation. Using a water bath, this study found a relatively low lethal temperature for *B. ramiflora* seeds, which survived half an hour's treatment at up to 50 °C, but neither blotted nor immersed seeds survived 60 °C and above. This similarity in immersed and blotted-dry seeds contrasts with *Piper aduncum*, an invasive species of forest margins in Xishuangbanna, in which the air-dried seeds can be heated for half an hour at 70 °C without significant viability loss, although most imbibed seeds also died at 50 °C (Wen et al. 2015). This difference between orthodox and recalcitrant seeds in tolerance to high temperature may be widespread in tropical plant species, including Mexican sunflower (Wen 2015; Table 2). Thus rapid viability loss of recalcitrant seeds from extreme high temperatures is very likely in fragmented rainforests, or after deforestation, since the maximum ground temperature may increase from about 25 °C in the rainforest interior to above 50 °C, even 70 °C on bare ground after deforestation.

Recalcitrant seeds can be neither air-dried nor heated, so they are more sensitive to increased temperature and decreased moisture, both of which result from rainforest fragmentation and deforestation. These seeds require the cool and moist habitat typical of rainforest interiors for germination and seedling establishment (Wen and Cai 2014). Rainforest fragmentation and deforestation caused more difficulties for recalcitrant-seeded species than orthodox-seeded species. As *B. ramiflora* seeds are only moderately to minimally recalcitrant, it can be expected that species with highly recalcitrant seeds, such as *Shorea chinensis*, *Vatica guanxiensis*, and *Pometia tomentosa*, which occur only in valleys, the moistest habitat in this area (Cao et al. 2008), will suffer even more than *B. ramiflora* from fragmentation and deforestation.

Table 2 Durable maximum temperature in seeds

Species name	Species type	Seed type	Durable maximum temperature (°C)	
			for blotted seeds	for imbibed seeds
Piper aduncum	invasive	orthodox	75	55
Tithonia diversifolia	invasive	orthodox	80	60
Ficus semicordata	pioneer	orthodox	80	65
Saprosma ternatum	climax	intermediate	55	55
Baccaurea ramifolra	climax	recalcitrant	57.5	55
Ardisia maculosa	climax	recalcitrant	55	50
Alocasis macrorrhiza	climax	recalcitrant	57.5	55

Data for *Tithonia diversifolia* and *Piper aduncum* cited from Wen (2015) and Wen et al. (2015), respectively

In conclusion, *B. ramiflora* seeds are sensitive to both dehydration and high temperatures stress although they are only moderately to minimally recalcitrant, and there is only a small discrepancy in high temperature tolerance between immersed and blotted-dry seeds. Moderate water and temperature stress inhibited germination while moisture contents below 18 % and temperatures above 50 °C are lethal. As recalcitrant seeds can be neither air-dried nor heated, species producing recalcitrant seeds have more difficulties in germination and seedling establishment in fragmented forests than those producing orthodox seeds. To prevent extinction of recalcitrant-seeded climax species from tropical rainforests, management should focus on maintaining interior forest conditions, facilitating their germination and seedling establishment.

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